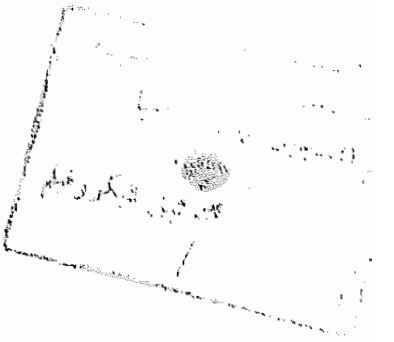
The Study Of Various Cytokines In Hyperthyroid States



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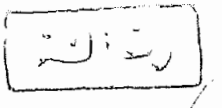

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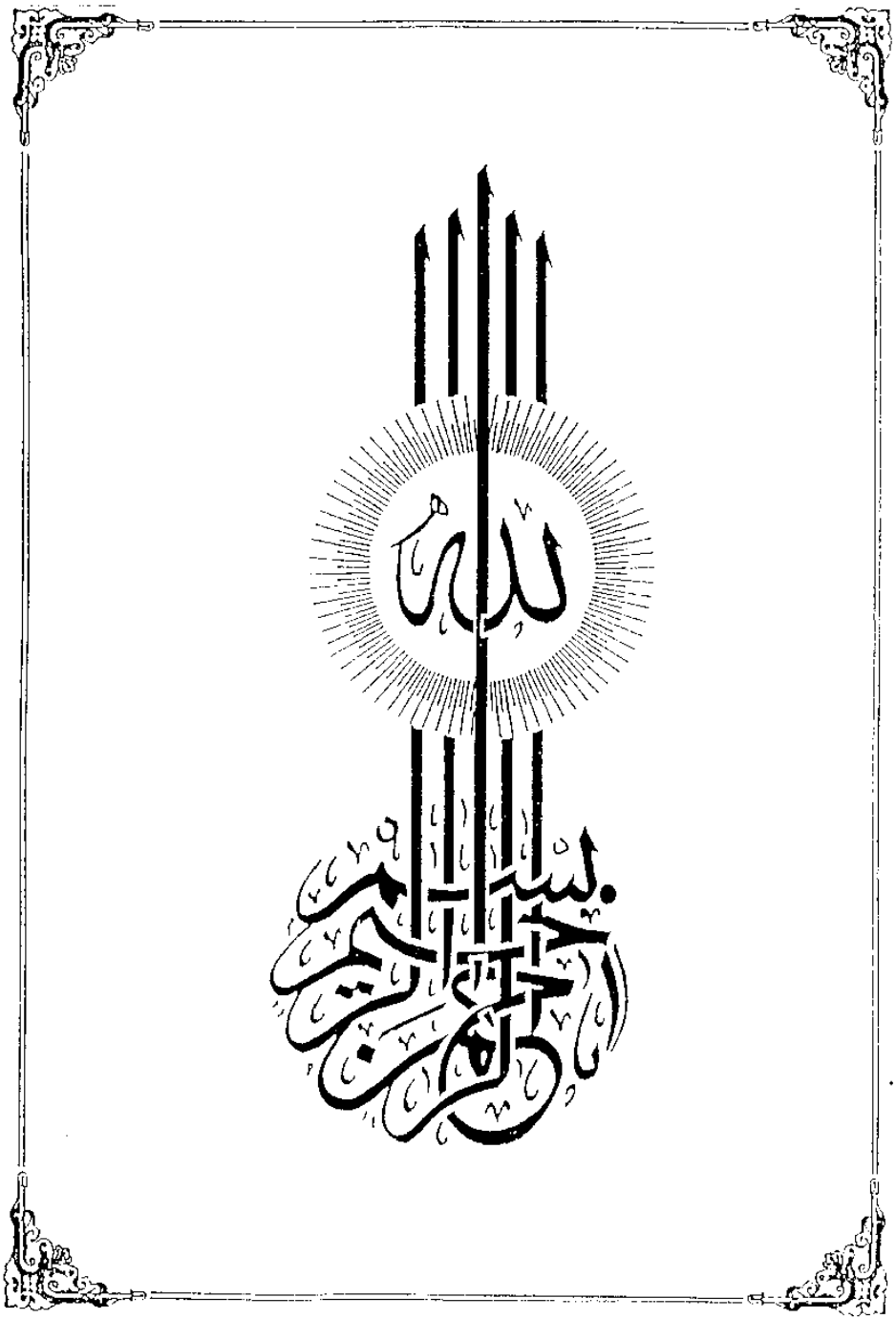

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The role of humoral immunity in thyroid disease has been studied extensively in the last decade. New interest among endocrinologists concerning the role of cell mediated immunity of hyperthyroid disease and its impact on the management of hyperthyroid diseases led us to study such relationship.

We had several goals along the course of the study:

- To proceed a step further in the clarification of the disturbance of cell mediated immunity in hyperthyroid states, through estimation of cytokines namely neopterin and interferon, bearing in mind the dilemma of whether it is a primary event with immunogenic background or a secondary phenomenon.
- To identify the relationship between the different cytokines in thyrotoxic states and a better understanding of these intracellular mediators in hyperthyroid states.
- To provide a new test to study the pathological state of the thyroid gland in autoimmune thyroid diseases. A test which would help to segregate queery cases of autoimmune thyroid diseases, predict clinical relapses and a marker for full patholcgical recovery.
- Lastly, to clarify the role of immunosuppression in the management of autoimmune thyroid states bearing in mind that antithyroid drug namely methimazole has anti T cell activity.

Review of Literature

Part I

THYROID AUTOIMMUNITY

Every individual possesses his own characteristic of histocompatibility antigens. This set is acquired by inheritance from the parents through the activities of genes that code for these antigens. These antigens are inherited together with the important antigens that influence immune responses through the activities of a complex set of genes known as the major histocompatibility complex (MHC) found on the short arm of chromosome 6 in men.

MHC molecules play an important role in immunoregulation and in the development of autoimmune diseases. The co-presentation of HLA class II molecules (DR, DP, DQ) with an antigen by presented macrophages is essential for the induction of T-helper cell function (Schleusener et al., 1989). Several groups have described this co-presentation of antigen not only by macrophages, but also by target cells, as for example, the thyroid cells and islet cells of the pancreas. It is, however not yet clear whether the presentation of the antigen by target cells is a primary or secondary event in the development of autoimmune thyroid diseases (Wick et al., 1987; Bottazo et al., 1983).

DEFINED THYROID SPECIFIC ANTIGENS

(Table 1)

Table 1: Thyroid specific antigens (Davies et al., 1986)

Name	Size	Cloned	Location	
			Primary Physical	Chromosome
Thyroglobulin	330kDa	Yes	Intra- and extracellular	8
Thyroid peroxidase	100kDa	Yes	Surface membrane	2
TSH receptor	80kDa	No	Surface membrane	-

Thyroglobulin:

Thyroglobulin (Tg) is a large glycoprotein dimer which is synthetized and secreted by the thyroid cell. Basal production of thyroid hormones results from pinocytosis of iodinated Tg and hydrolysis by lysosomal enzymes. Post-translational modifications, together with the degree of Tg iodination are important determinants of the immunogenecity of the thyroglobulin (Tg) molecule (Champion et al., 1987).

The intact human Tg mRNA contains 8448 nucleotides plus a polytail (coding for 2748 residues with 72 tyrosines plus a 19 signal sequence) and the primary structure

of this large human gene has been determined on chromosome 8 (Malthiery and Lissitzky, 1987).

In thyroid stimulating hormone (TSH) activated thyroid cells, mRNA coding for Tg represents about 10% of total RNA polymerase II activity. The close proximity of the Tg gene to the c-myc oncogene has suggested that gene rearrangements may put the c-myc oncogene under the control of the strong Tg promoter in thyroid cancers (Wassart et al., 1985).

Thyroid peroxidase - the microsomal antigen:

Thyroid peroxidase (TPO) is responsible for the iodination of tyrosine residues on Tg and the intramolecular coupling reaction of iodinated tyrosines leading to the formation of thyroxine (T_4) and triiodo thyronine (T_3). TPO is present on the thyroid cell surface and is another important antigenic target in autoimmune thyroid disease TPO has a molecular weight of about 100 kDa and has been identified as the characterized thyroid microsomal antigen (Seto et al., 1987).

The TPO molecule is synthesized as a single polypeptide by a gene localized to the short arm of chromosome 2. TPO mRNA, like Tgm RNA, is under thyroid stimulating (TSH) control (Kimura et al., 1987).

TSH receptor structure

TSH receptor binding protein is present in very low quantities on the thyroid cell surface and consists of two subunits linked by a disulphide bond (Rees Smith et al., 1988). Similar binding sites have been located on adipose tissue, testis tissue and lymphocytes (Davies et al., 1978). The physiological relevance of these latter sites is uncertain, although TSH secretion by immune cell lines suggests the presence of an intra-immunological feed back loop. The binding of TSH to its receptor is highly influenced by increases to ionic strength which may relate to the non-covalent binding of the subunits. When TSH, which has an elongated structure combines with the 50 Kda subunit, which has a compact structure, an elongated structure is formed which may enhance its antigenicity (Rees Smith et al., 1988). The mechanism by which message transfer through the receptor molecule occurs is unknown but the receptor is G protein-linked and utilizes cyclic AMP and the phosphoinositol pathway as a second messenger. Sequence data on TSH receptor, or any other large glycoprotein hormone receptor, are not yet available. The TSH receptor has not been expressed in a functional form from an isolated gene.

Orbital Antigens:

Much controversy exists over the presence of specific retro-orbital antigens which may be associated with ophthalmic Graves' disease, as evidenced by antibodies binding to retro-orbital tissue (Kodama, 1982) and T cell interaction with muscle cells (Kriss and Mahdi, 1979). Cross-over specificity between acetyl cholinesterase and Tg has been claimed on the basis of sequence sirilarities (Ludgate et al., 1986), and monoclonal antibodies have been generated demonstrating such cross-over specifity. However, the lack of clear evidence of muscle pathology as a primary event in the disease suggests that differentiating retro-orbital fibroblasts from peripheral fibroblasts may also offer new insights into retro orbital antigenic targets (Davies et al., 1986).

THYROID ANTIGEN-SPECIFIC AUTOANTIBODIES

Animal studies have shown selective use of certain genes for autoantibody production (Painter et al., 1986). Genetic studies, in human autoimmune thyroid disease have been hampered by the polyclonicity of the responses and the difficulty in immortalizing human thyroid antibody secreting B cells. Some data relate IgG heavy chain markers (Gm) assessed serologically, to familial autoimmune thyroid disease (Uno et al., 1981).

Autoantibodies to Tg (Tg-Ab) and TPO (TPO-Ab)

Autoantibodies to Tg and TPO antigens have been studied for many years. There is a clear association of higher titres of high affinity IgG antibodies with autoimmune thyroid disease, particularly autoimmune thyroiditis. (Janson et al., 1986). Their role in the primary aetiology of such disease, rather than as part of a secondary response, remains unclear. There is a wealth of data demonstrating that such antibodies can be involved in antibody dependent T cell killing as well as direct lysis by complement fixation. IgG, subclass correlates with higher anti-TPO titres and possibly

more predictable thyroid failure (Davies et al., 1986, Janson et al., 1986).

As the methods for detecting thyroid autoantibodies have become more sensitive, typically using ELISA techniques, their clinical relevance has been questioned, since up to 25% of the normal population may have evidence of anti-Tg or anti-TPO in their circulation (Roman et al., 1984). Furthermore, thyroid autoantibodies which are of IgM subclass have been reported as inducible in a large population, if not all, of the general population (Iitaka et al., 1988). It has beenshown that human monoclonal antibodies of the IgM class which recognize thyroid antigens may also show a variety of cross-over specificities (Ternynek and Avramess, 1986). Nevertheless, it is possible that such autoantibodies are important in the normal immune surveillance systems of the body and that a class switch from IgM to IgG is the hall mark of a pathological response to excess antigen.

TSH receptor antibodies (TR-Ab)

The receptor antibodies may act as TSH agonists or antagonists when they interact with the TSH receptor molecule. The classical TRAbs remain the thyroid stimulating antibodies of hyperthyroid Graves disease. In this